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Original Article

Newborn screening for cystic fibrosis in Switzerland — Consequences after analysis of a 4 months pilot study☆

Toni Torresani [a], Ralph Fingerhut [a], Corina S. Rueegg [b], Sabina Gallati [c], Claudia E. Kuehni [b], Matthias R. Baumgartner [d], Juerg Barben [e,*]The Swiss CF Screening Group [1]

[a] Swiss Newborn Screening Laboratory, University Children's Hospital, Zürich, Switzerland
[b] Institute of Social and Preventive Medicine (ISPM), University of Bern, Bern, Switzerland
[c] Division of Human Genetics, University Children's Hospital, Bern, Switzerland
[d] Division of Metabolism and Swiss Newborn Screening, University Children's Hospital, Zürich, Switzerland
[e] Division of Paediatric Pulmonology, Children's Hospital, St. Gallen, Switzerland

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Abstract

Background: Switzerland introduced newborn screening (NBS) for CF in 2011, using an IRT/DNA/IRT protocol. This paper describes the results of the first year and compares two versions of the protocol with different IRT cut-offs, particularly effects on recall rate, sensitivity and specificity.
Methods: IRT cut-offs were >45 ng/ml (99.0th percentile) in period 1 (months 1–4) and >50 ng/ml (99.2nd percentile) in period 2 (months 5–12). In period 2 we abstained from recalls when none of the 7 most common CF mutations were detected and IRT was <60 ng/ml.
Results: In periods 1 and 2, 26,535 and 56,663 tests were performed. Recall rates were 0.94% and 0.48%, respectively ($p < 0.001$), PPV increased from 23% to 47% ($p = 0.024$) and sensitivity was 90% and 100%.
Conclusions: Raising initial IRT cut-off from the 99.0th to the 99.2nd percentile and abstaining from recalls for children with an IRT < 60 ng/ml and carrying no major CFTR mutation significantly reduced the recall rate without affecting sensitivity.

Keywords: Newborn screening; Cystic fibrosis; Immunoreactive trypsinogen; Recall rate

1. Introduction

In Switzerland, newborn screening (NBS) was introduced in 1965 for the detection of Phenylketonuria, using a dried blood-spot (Guthrie-test) on the 4th day of life. In the meantime, five diseases were added to the NBS. Since 2006, NBS was centralised and around 80,000 tests per year were performed by the Swiss Newborn Screening Laboratory (SNSL). In January 2011, NBS for cystic fibrosis (CF) was introduced as a 2-year pilot study, after conducting a retrospective study which confirmed that the planned screening protocol with immunoreactive trypsinogen (IRT) and the seven most common CF mutations in the Swiss population would have detected all clinically diagnosed children with classic CF in the years 2006–2009 included in this study [1,2]. For the pilot study, we chose the 99th percentile for the initial IRT cut-off as used in many NBS programmes [3,4].

Since the implementation of a new law on genetic testing in Switzerland in 2007, written informed consent is mandatory for genetic tests in the CF-centres; but not for the NBS [1]. After delivery, all families get a brochure explaining NBS and the currently screened diseases (www.neoscreening.ch); if they do

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* Corresponding author at: Paediatric Pulmonology, Children's Hospital, CH-9006 St. Gallen, Switzerland. Tel.: +41 71 243 71 11; fax: +41 71 243 76 99.
E-mail address: juerg.barben@kispisg.ch (J. Barben).

[1] Constance Barazzone, Carmen Casaulta, Anne Mornand, Peter Eng, Gaudenz Hafen, Jürg Hammer, Alexander Möller, Dominik Müller, Nicolas Regamey, Isabelle Rochat, Barbara Schiller, Martin H. Schöni, Renate Spinas, Johannes Spalinger, Daniel Trachsel, Maura Zanolari.

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not actively disagree, their baby will be tested. In case of a positive NBS result, the SNSL informs the nearest CF-centre, which invites the child for further investigations. According to the new law, the SNSL is not allowed to provide the CF-centres with the exact genotype.

American and European guidelines for implementing NBS for CF recommend careful evaluation of every country's screening protocols to achieve optimum sensitivity and positive predictive rates [5,6]. In the first year of the pilot study, we therefore tested two subsequent versions of the protocol, using each a different IRT cut-off (99th versus 99.2nd percentile) and a different recall programme for a second heel-prick test. The goal of this paper was to determine which protocol was most effective. We compared recall rate, sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) between the first period (months 1–4; IRT: 99th percentile) and the second period (months 5–12; IRT: 99.2nd percentile).

2. Methods

2.1. General design of the Swiss CF-NBS

The protocol of the CF-NBS was divided into two parts: The first part was the screening tests, performed by the Swiss Newborn Screening Laboratory (SNSL) at the University Children's Hospital in Zürich (see Investigations in the SNSL). In the SNSL, the CF protocol begins with an initial IRT measurement in a dried blood-spot, from a heel-prick sample at the 4th day of life (see IRT measurement [1st tier]). The blood-spots are sent by the birth clinics to the SNSL. A genetic screening testing a limited number of mutations was performed in the same blood-spot if initial IRT was above the level of the cut-off (see CFTR mutation screening [2nd tier]). If no mutation was found, a second heel-prick test was required to repeat measurement of IRT. For this reason, the SNSL informed the birth clinic, and they organized a second heel-prick test by the midwife or the paediatrician two weeks later. Children who were positive were referred to a CF-centre if they had either an elevated level of initial IRT and at least one CF mutation, or showed elevated IRT after two screenings.

The second part of the screening protocol was diagnostic evaluation of all children who had tested positive. These were carried out in one of eight specialized Swiss paediatric CF-centres (see Investigations in the CF-centres). All children referred to CF-centres were given diagnostic sweat tests (see Sweat test). If these were positive, borderline or inconclusive, another blood sample was taken so that a detailed genetic analysis could be performed in the genetic reference laboratory at the University of Bern (see CFTR mutation analysis). In addition, faecal elastase was measured to assess pancreas functionality at the time of the sweat test.

To aid in determining the sensitivity of the newborn screening protocols, all paediatric CF-centres agreed to report to the study centre any new CF cases that were diagnosed outside the screening programme.

2.2. Investigations in the SNSL

2.2.1. IRT measurements (1st tier)

IRT is measured in dried blood spots using the GSP Neonatal IRT-Kit (PerkinElmer Wallac, Turku, Finland) [7–9]. Analytical sensitivity of the assay is approximately 3.0 ng/ml blood. Intra- and inter-assay variation for the clinically relevant area is less than 6%.

2.2.2. CFTR mutation screening (2nd tier)

The SNSL determined the seven most common CFTR mutations in Switzerland with an in-house developed kit (SWISS PANEL: F508del, 3905insT, G542X, R553X, W1282X, 1717-1G>A, N1303K) [10,1]. For the first 4 months, the SNSL used in addition to the in-house kit a commercial CFTR mutation kit (LUMINEX xTAG® Cystic Fibrosis 39 kit v2, Luminex Corporation, Austin, USA), which tested for 39 mutations, as it was not guaranteed that the components of the in-house kit would be available in the future.

LUMINEX is based on a Multiplex PCR amplification of DNA, followed by fluorescent label incorporation using analyte specific primer extension (ASPE). The ASPE product is hybridized to beads. Results are returned by fully automated process that uses software specifically designed for the task.

2.3. Investigations in the CF-centres

2.3.1. Sweat tests

Two sweat tests are performed simultaneously for each child, using the Macroduct® and Nanoduct® systems. The Macroduct®-system uses pilocarpine iontophoretic stimulation (Webster Sweat Collection System 3700-SYS, Wescor), followed by sweat collection and sweat chloride concentration measurement in the laboratory [11]. A minimum of 15 µl sweat is required to determine chloride level. Sweat chloride of >60 mmol/l is considered positive for CF; <30 mmol/l is normal [12]. Values between 30 and 60 mmol/l were borderline and these children were further assessed according to the current guidelines for equivocal CF [13]. The Nanoduct®-system assesses conductivity [14]. Its continuous sweat flow sensor requires only 3–5 µl of sweat. Sweat conductivity is approximately 15 mmol/l higher than sweat chloride, so a value of >80 mmol/l is consistent with the diagnosis of CF; a value of <50 mmol/l is normal [14].

2.3.2. CFTR mutation analysis

The genetic reference laboratory at the University of Bern performed diagnostic genetic testing. All parents provided written informed consent for CFTR gene analysis. Genomic DNA was extracted from peripheral blood cells using standard procedures. In a first step, the laboratory tested for a panel of 32 mutations, using an in vitro diagnostic device (CF PCR-OLA kit, v.3, Abbott AG) based on multiplex PCR amplification in combination with an oligonucleotide ligation assay (OLA) that produced allele-specific fluorescent labelled fragments, which were separated by capillary electrophoresis. When the device detected 0 to 1 mutation in a child, we proceeded to screen the entire coding sequence of the CFTR gene, including intron/

exon boundaries and the promoter region, using the single-strand conformation polymorphism/heteroduplex technique, with a sensitivity of 97–98% [15] followed by direct sequencing of the variants (ABI 377 sequencing system, Applied Biosystems, USA). Multiplex Ligation-dependent Probe Amplification (SALSA MLPA probe mix P091-C1 CFTR-v07, MRC Holland) was used to test for large deletions and duplications.

2.4. Detailed protocol of period 1 (months 1–4)

In the literature, the most widely used cut-off value for the IRT is the 99th percentile (approximately 60 ng/ml) [4]. However, in a test run using blood spots collected in December 2010 in Switzerland, the cut-off for 99th percentile was 45 ng/ml [1]. We therefore used a 45 ng/ml cut-off for the first period. For DNA screening in the first period, we used both the SWISS PANEL and LUMINEX assays in parallel (see CFTR mutation screening (2nd tier)). Children with ≥1 detected CFTR mutation were referred to a CF centre. We requested a second heel prick test for a repeat IRT measurement for infants in whom CFTR mutation was not detected. Infants whose second IRT was >45 ng/ml were also referred to a CF centre.

After 4 months, we examined initial and repeat IRT values, and data from the genetic screening to assess the results (Tables 1 and 2). None of the CF cases had an initial IRT < 63 ng/ml. None of the 15 children who had two elevated IRTs but no CFTR mutation had classical CF. Based on these results, we adapted the protocol for the second period in four different ways, described below.

2.5. Detailed protocol of period 2 (months 5–12)

First, we increased the initial IRT cut-off from 45 ng/ml (99th percentile) to 50 ng/ml (99.2nd percentile). Second, we decided not to give a second heel prick test to children whose initial IRT was <60 ng/ml and who had no SWISS PANEL mutation. Third, we stopped using the LUMINEX kit for genetic screening. Fourth, in children with meconium ileus (MI), CFTR mutation screening was always performed irrespective of the IRT value.

2.6. Statistical analysis

We described the results of the screening and the diagnostic evaluation for all children referred to a CF centre during the two periods. Then we calculated the effectiveness of the screening tests for the first and second periods, comparing recall rate, sensitivity, specificity, PPV and NPV. Finally, we made a frequency distribution of the initial IRT values of all newborns in 2011, to see the effect of the different cut-off levels.

3. Results

3.1. Period 1 (months 1–4)

Between January and April 2011, 26,535 IRT tests were performed; 250 (0.94%) samples had values > 45 ng/ml and led to DNA screening (Fig. 1, Table 1). Twenty-four children, with one or two mutations, were directly referred to a CF-centre. In 226 children, IRT was measured again by a second heel prick (recall rate = 0.85%). In 15 of these children, IRT was still >45 ng/ml and they were referred to a CF centre. One child (case No. 7, Table 2) was referred to the CF centre despite a second IRT value of <45 ng/ml (43.6 ng/ml) because we were cautious in the early phase of screening and the decrease in IRT after the second test was small. Of the 24 children directly referred, 20 had ≥1 SWISS PANEL mutation, and four had a mutation detected only by the LUMINEX test (Table 2).

In total, 39 children were examined in a CF centre (referral rate = 0.15%) at a median time of 30 days (range: 12–151). In

Table 1
First year of CF-NBS in Switzerland: comparison of different outcome parameters between period 1 (months 1–4) and period 2 (months 5–12).

Parameter	Months 1–4 (N = 26,535)			Months 5–12 (N = 56,663)			
	n	N	%	n	N	%	p-Value [a]
1st IRT above cut-off	250	26,535	0.94	397	56,663	0.70	<0.001
At least one CFTR mutation detected	24	250	9.60	41	397	10.33	0.157
Recall rate for 2nd heel prick test (2nd IRT measurement)	226	26,535	0.85	230	56,663	0.41	<0.001
Overall recall rate (2nd heel prick test and referral to CF centre)	250	26,535	0.94	271	56,663	0.48	<0.001
Referrals to CF-centre	39	26,535	0.14	45	56,663	0.08	0.002
Classic CF	7	26,535	0.03	20	56,663	0.04	0.499
Equivocal CF	2	26,535	<0.01	1	56,663	<0.01	0.203
Sensitivity (all CF)	9	10 [b]	90.00	21	21	100.00	0.141
Specificity (all CF)	26,495	26,525	99.89	56,618	56,642	99.96	<0.001
Positive predictive value all CF (based on recall)	9	250	3.60	21	397	7.75	0.042
Positive predictive value all CF (based on referral)	9	39	23.08	21	45	46.67	0.024
Positive predictive value only classic CF (based on recall)	7	250	2.80	20	397	5.04	0.172
Positive predictive value only classic CF (based on referral)	7	39	17.94	20	45	44.44	0.005
Negative predictive value	26,495	26,496	99.99	56,618	56,618	100.00	0.017

Abbreviations: CF, Cystic Fibrosis; CFTR, Cystic Fibrosis Transmembrane Conductance Regulator; IRT, Immunoreactive Trypsinogen.
[a] p-Value calculated from two-sample proportion test.
[b] Including one clinically diagnosed child with meconium ileus (IRT 39.5 ng/ml).

Table 2
Characteristics of all children, who were referred to a CF centre for diagnostic testing within 4 months (n = 40).

Case no	GA (weeks)	Weight at birth (g)	1st IRT (4th day of life) (ng/ml)	2nd IRT (repeat heel prick) (ng/ml) [a]	CFTR mutation screening SWISS PANEL in NBS laboratory [b]	CFTR mutation screening LUMINEX in NBS laboratory [b]	CFTR mutation diagnosis in genetic reference laboratory [c]	Sweat chloride macroduct method (mmol/l) [d]	Sweat conductivity nanoduct method (mmol/l) [d]	Pancreas function faecal elastase (ng) [e]	Diagnosis
1	39	2980	183.0		F508del/–	F508del/–	F508del/420del9	97	113	45	CF
4	39	3290	150.2		F508del/F508del	F508del/F508del	F508del/F508del	–	129	23	CF
11	37	3400	270.0		F508del/–	F508del/–	F508del/2347delG	105	98	<15	CF
14	40	3655	114.4		F508del/F508del	F508del/F508del	F508del/F508del	–	116	37	CF
21	37	3280	119.5		F508del/F508del	F508del/F508del	F508del/F508del	ND	ND	<15	CF with MI
23 [f]	38	2900	76.9		F508del/F508del	F508del/F508del	F508del/F508del	ND	ND	<15	CF
35	37	2930	134.8		F508del/–	F508del/621+1G>T	F508del/621+1G>T	110	139	<15	CF
40	37	2980	39.5	41.8	F508del/F508del	F508del/F508del	F508del/F508del	–	97	<15	CF with MI
12	41	3810	65.9		–/–	R347P/–	R347P/4006-46del5 [h]	38	37	ND [i]	Equivocal CF
20 [g]	38	2720	63.0	122.9	–/–	–/–	T5/T1086A [h]	35	–	382	Equivocal CF
2	41	3250	50.1		F508del/–	F508del/–		–	39		No CF
10	38	2590	51.3		F508del/–	F508del/–		14	47		No CF
13	39	3330	68.8		F508del/–	F508del/–		11	36		No CF
17	39	2670	64.1		1717-1G>A/–	1717-1G>A/–		20	15		No CF
18	40	3360	58.9		3905insT/–	3905insT/–		13	36		No CF
22	38	2970	51.3		F508del/–	F508del/–		–	27		No CF
24	36	2790	49.1		F508del/–	F508del/–		10	48		No CF
27	40	3420	60.7		F508del/–	F508del/–		6	23		No CF
31	40	4400	55.5		F508del/–	F508del/–		14	29		No CF
32	41	4460	89.5		F508del/–	F508del/–		14	33		No CF
33	40	3700	130.6		F508del/–	F508del/–		ND	36		No CF
34	40	3005	65.4		N1303K/–	N1303K/–		24	37		No CF
36	39	2780	61.5		F508del/–	F508del/–	F508del/– [d]	ND	–		No CF
15	40	3310	49.3		–/–	R347H/–		10	39		No CF
16	37	3240	56.0		–/–	R347H/–		18	54		No CF
29	34	1870	126.5		–/–	2184delA/–	2184delA/–	–	–		No CF
3	41	3860	46.2	72.6	–/–	–/–		13	44		No CF
5	37	2840	60.1	61.0	–/–	–/–		–	51/34		No CF
6	40	3030	56.6	56.2	–/–	–/–		16	34		No CF
7	37	3130	49.0	43.6	–/–	–/–		6	18		No CF
8	39	4320	48.7	94.9	–/–	–/–		13	27		No CF
9	37	2050	127.9	52.3	–/–	–/–		28	28		No CF
19	38	3570	68.1	54.5	–/–	–/–		6	45		No CF
25	35	2300	61.5	50.2	–/–	–/–		12	37		No CF
26	40	2780	58.2	59.8	–/–	–/–		10	48		No CF
28	40	3430	56.5	53.4	–/–	–/–		13	31		No CF
30	37	2930	54.2	65.6	–/–	–/–		–	32		No CF
37	40	3615	65.9	191.4	–/–	–/–		ND	51		No CF
38	41	4350	56.2	65.1	–/–	–/–		ND	41		No CF
39	42	2900	51.6	68.5	–/–	–/–		20	–		No CF

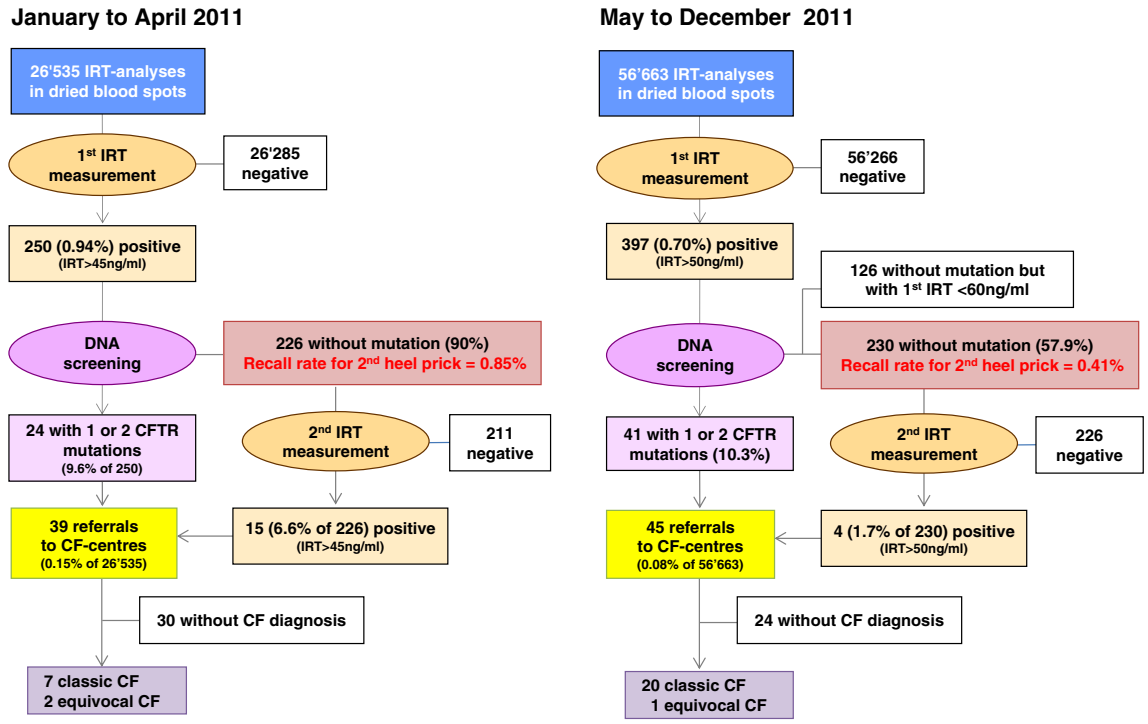

Fig. 1. Evaluation of the first year of the CF newborn screening in Switzerland. Abbreviations: CF, Cystic Fibrosis; CFTR, Cystic Fibrosis Transmembrane Conductance Regulator; DNA, Deoxyribonucleic Acid; IRT, Immunoreactive Trypsinogen; PPV, Positive Predictive Value.

nine, CF diagnosis was confirmed either by a positive sweat test and/or two CFTR mutations (PPV = 23.1%; Table 1). Seven children had a classic CF carrying at least one of the SWISS PANEL mutations; four were homozygous, and three were heterozygous for F508del. Two children carrying one CFTR mutation not associated with classic CF (equivocal CF) were both pancreatic-sufficient and asymptomatic by age one year (case Nos. 12 and 20, Table 2).

All seven children with classic CF and the two children diagnosed with equivocal CF had an initial IRT value of >60 ng/ml (range: 63–270 ng/ml, Table 2). All 14 children with initial and repeat IRT between 45 and 60 ng/ml, but no SWISS PANEL mutation, had normal sweat tests or no CFTR mutations after extended gene analysis. One false negative IRT result was detected in a child clinically diagnosed because of a MI. This child was homozygous for F508del, but had a normal IRT (39.5 ng/ml, case No. 40, Table 2).

3.2. Period 2 (months 5–12)

In the second period, 56,663 IRT tests were performed and 397 (0.70%) were >50 ng/ml (Fig. 1, Table 1). In 41 of these children, at least one SWISS PANEL mutation was detected. Of the remaining 356, 126 had an initial IRT < 60 ng/ml. Therefore, a second IRT test was performed for only 230 children, leading to a decreased recall rate for a second heel prick test by 52% (0.85% vs. 0.41%). Of the 230 children, only four had an elevated repeat IRT and were referred to a CF centre. One of these four children was diagnosed with CF (heterozygous 711+5G>A/3659delC, sweat chloride 82 mmol/l, pancreas-insufficient, initial IRT 207 ng/ml); the other three were healthy. The CF centres have so far found no children with CF who were not detected by the screening procedure (with an initial IRT < 50 ng/ml). Of the 45 children referred (referral rate 0.08% compared to 0.15% in period 1), 20 were

Notes to Table 2:
Abbreviations: CF, cystic fibrosis; CFTR, Cystic Fibrosis Transmembrane Conductance Regulator; GA, Gestation Age; IRT, Immunoreactive Trypsinogen; MI, Meconium Ileus; mts, months; NBS, Newborn Screening; ND, not done; No, Number.

[a] No 2nd IRT test was done in children where at least one CFTR mutation was detected in the screening.
[b] A dash in the columns of the genetic analyses means that the investigation was done but no CFTR mutation was detected.
[c] CFTR mutation diagnosis was only done in children with positive, not valid or inconclusive sweat test.
[d] A dash in the columns of the sweat tests means that the test was attempted but the result was not valid (not enough sweat, technical problems).
[e] Faecal elastase was determined in children with CF or equivocal CF at the first assessment. If it was normal (>200 ng), the measurement was repeated in the first year of life.
[f] This child had also a Trisomy 21 and an atrium septum defect (ASD) and was on the intensive care unit because of a neonatal respiratory distress syndrome.
[g] This child had also a complex heart disease and renal insufficiency.
[h] This CFTR mutation is so far not described as CF-causing mutation.
[i] The parents refused to determine the faecal elastase, but the stool quality and the child's growth were normal after one year of life.

diagnosed with CF and the genotype of one child met the criteria of equivocal CF (Table 1, Online-Table 3). This corresponds to a PPV of 47%, compared to 23% in the first period. All children were seen in the CF centre at the median time of 22 days (range: 4–314). In period 2, we had 5 children with MI; 4 had an IRT > 50 ng/ml (range: 77.7–102.3), and one below the 99.2nd percentile (42.9 ng/ml).

4. Discussion

Two changes to the initial protocol (increasing the initial IRT cut-off from 45 to 50 ng/ml and abstaining from a second IRT test if the initial IRT was <60 ng/ml) significantly cut down recalls without reducing sensitivity (e.g. missing a child with CF). Because the goal of an NBS is not only to detect as many cases as possible, but also to minimize false positive results, these results are important. Initial IRT cut-off has a powerful effect on the effectiveness of NBS, determining the sensitivity and specificity of a programme [5]. Proper adjustment of the initial IRT cut-off addresses the following problems.

False positive results and second heel prick tests pose a challenge to NBS programmes as they bring families to unnecessary medicalization. In addition, when non-affected children with elevated IRT are tested in a CF centre, their parents may feel anxious or depressed while awaiting definitive results [16]. In a minority of cases, parental anxiety may persist for some time, despite subsequent negative sweat test results [17]. Thus we wished to minimize false positives and unnecessary recalls, while preserving diagnostic sensitivity through close monitoring of the implementation of the CF-NBS. A recall rate of 0.85% for a second heel prick test within the first four months prompted us to reassess our strategy and optimize initial IRT cut-off.

In the first period, no child with an initial IRT of <60 ng/ml had CF (Fig. 2). Because the evaluation period (four months) was short, we were conservative and chose the cut-off of 50 ng/ml (99.2nd percentile) rather than 60 ng/ml (99.5th percentile). In fact, in the second period we did detect one CF case with an IRT of 59 ng/ml. By increasing the cut-off for genetic testing to 50 ng/ml, and abstaining from a second IRT test if the initial IRT was <60 ng/ml, we lowered recall rate for a second heel prick test by 52% (0.85 vs. 0.41; $p < 0.001$; Table 1), specificity increased from 99.89% to 99.96% ($p < 0.001$), and the PPV improved from 23% to 47% ($p = 0.024$). In the second period, only 4 children with persistently elevated IRTs but no CFTR-mutation were referred to a CF centre. One of these was diagnosed with CF with two rare CF mutations in Switzerland. This child had an initial IRT of 207 ng/ml (>99.9th percentile; Fig. 2).

Safety net strategies for NBS programmes are currently debated. The Australian experience of CF-NBS demonstrated that additional CF testing for infants with an elevated IRT but no CFTR mutation has an extremely low yield, regardless of the IRT level [18]. To ensure that we do not miss children with CF carrying mutations uncommon in Switzerland, we initially performed a second IRT in all infants with elevated initial IRT where no CFTR mutation was detected. After 4 months, we developed a safety net (Fig. 3), in which only children with an elevated IRT > 60 ng/ml but without any CFTR mutation receive a second IRT measurement. In period 2 of our study, one out of four children, who was referred to a CF centre because of a second elevated IRT (1/230 = 0.4%, Fig. 1), had a classic CF. At the end of the two-year pilot, we will decide whether to continue to recall children who have an elevated IRT > 60 ng/ml but no CFTR mutation or increasing the IRT threshold that triggers a second IRT in order to further reduce a second heel prick test. A possible alternative is to change our protocol and create a different safety net; e.g., refer all children with an IRT > 99.9th percentile whether or not a CFTR mutation is detected. This would enable infants to avoid a second heel prick test.

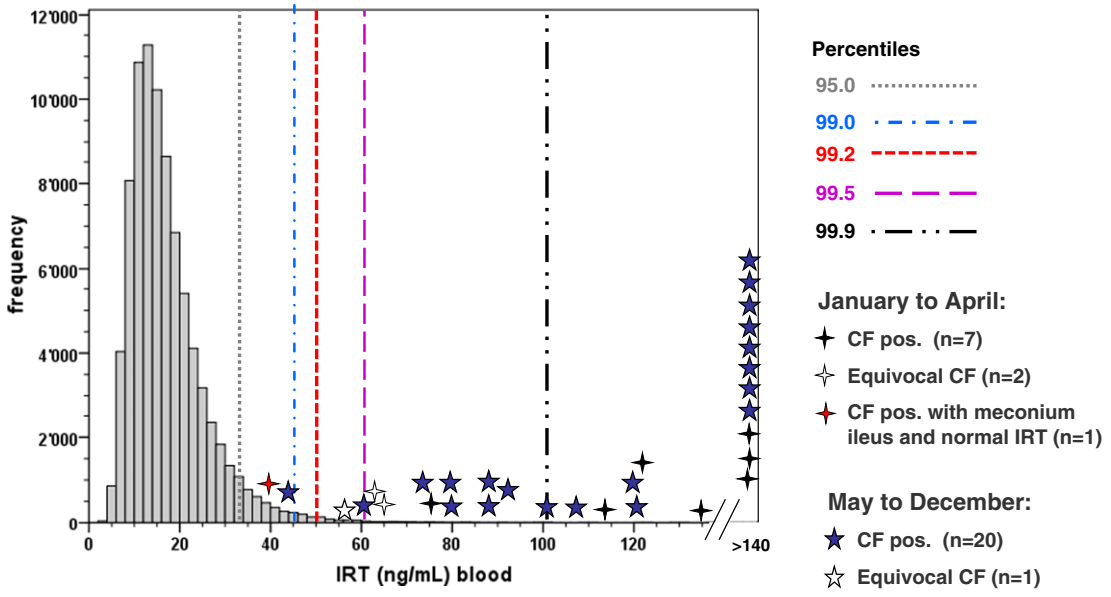

Fig. 2. Frequency distribution of initial IRT values in 2011. Abbreviations: CF, Cystic Fibrosis; IRT, Immunoreactive Trypsinogen.

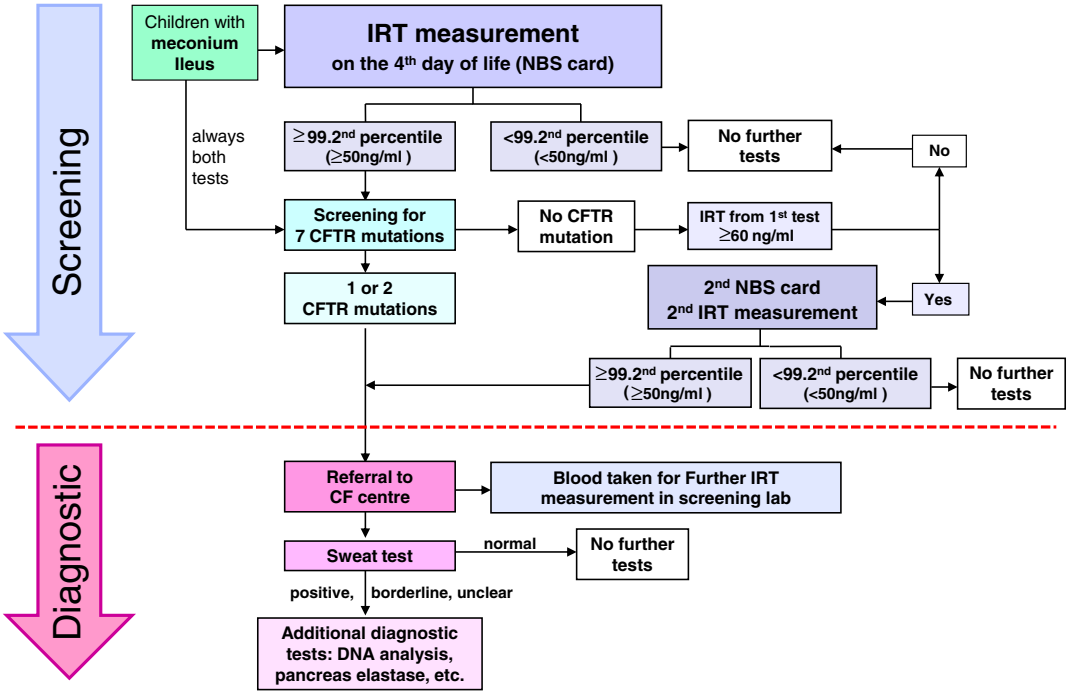

Fig. 3. New algorithm of CF newborn screening in Switzerland since May 2011. Abbreviations: CF, Cystic Fibrosis; CFTR, Cystic Fibrosis Transmembrane Conductance Regulator; IRT, Immunoreactive Trypsinogen; NBS, Newborn Screening.

All NBS programmes aim for low levels of false negative results. The screening programmes from Australia have a false negative rate of up to 8%, and up to 25% of infants with MI have a normal IRT [3,8,19,20]. Therefore, high risk infants such as newborns with MI and sibling with CF should always have a sweat test, regardless of the screening results [19]. In the first four months, there were two infants with CF and MI, but only one was detected by our screening protocol. This explains the sensitivity of 90% in the first period. We then adapted our protocol and used direct CFTR mutation screening in all children with MI irrespective of the IRT value (Fig. 3), and we had no further false negatives. However, final evaluation of sensitivity takes years because children with mild CF develop symptoms only later in life.

The strength of this study is our careful documentation of the results of each step of the CF-NBS. We analysed the results of each screening step in detail and thoroughly documented the effects of changes to the protocol. Involvement of all Swiss CF centres and independent assessment of clinically diagnosed CF cases allowed us to carefully monitor sensitivity. However, the duration of the two periods was relatively short; this limited statistical power. During the first period, 39 children were referred to a CF-centre and only seven were diagnosed with classical CF. These are low numbers as basis for adapting the protocol. Continuous monitoring over the next years will allow us to make more precise estimates and derive more robust conclusions.

In conclusion, changes in the protocol implemented during the first year of the Swiss NBS for CF resulted in significantly fewer recalls and higher PPV, without a loss in sensitivity. A small protocol change, even after a short period, can have profound impact on the performance of a programme reducing the unnecessary medicalization of a large number of families and the negative impacts this has on their well-being. This study highlights that NBS needs to be monitored closely and programmes need to be responsive to the results and not accept high levels of recall and sweat testing.

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Competing interests

There are no competing interests.

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